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## AN UPDATE

- Guidelines for setting up of a Molecular Laboratory

## DRUG UPDATE

- Gefapixant

## CASE REPORT

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- Management of a Symblepharon Case by Auto Conjunctival Graft: A Clinical Case Report



# The Rajasthan Medical Journal

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## ORIGINAL ARTICLE

# Study of Catheter-Associated Bloodstream Infection with Non-tunneled Hemodialysis Catheter and its Clinical Implication.

Shashank Bhardwaj\*, Seetaram Singh\*, Tushar Gupta\*, Dhananjai Agrawal\*\*, Vinay Malhotra\*\*, Rakesh Gupta\*\*\*, Gigin SV\*, Kavish Sharma\*, Niranjani Gogoi\*, Shivi\*\*\*\*

### ABSTRACT

**Background:** The preferred modality of vascular access for hemodialysis is an arteriovenous fistula but Non Tunneled Hemodialysis catheters (NTHCs) remain the preferred vascular access for hemodialysis (HD) initiation in developing countries. Central-venous-catheter-associated bloodstream infection (CABSI) is an important cause of hospital-acquired infection associated with morbidity, mortality, and cost.

**Study objective:** To study the incidence and microbiological spectrum of NTHC-associated bloodstream infections (CABSIs) at a tertiary care center.

**Design:** Prospective cohort study.

**Sample:** All adult ( $\geq 18$  years) hemodialysis patients who underwent NTHC insertion & who met study protocol were approached for inclusion in this study.

**Duration of study:** One year.

**Results:** Data from 171 patients was analyzed in this study. Out of 171, 40 patients (23.3%) developed CABSI. Out of all the male patients, 24.2% developed CABSI, while 22.2% females developed CABSI. On statistical analysis, no significant difference was found in age and gender distribution for developing CABSI in this population. Hospital stay increased significantly in those patients who developed CABSI, with a mean duration of  $19.00 \pm 7.09$  days ( $P < 0.001$ ). This study showed a statistically significant association between development of CABSI and comorbidities like diabetes, hypertension, anemia and hypoalbuminemia. Overall mortality in this population was 7% (12/171). Out of 40 patients who had

CABSI, 9 died (22.5%). Chi-square test showed a significant correlation of CABSI with mortality ( $p < 0.001$ ).

**Conclusion:** Non Tunneled Hemodialysis catheters (NTHC) associated CABSI occur at a high rate with significant morbidity and mortality, especially in diabetics, hypertensive and those with anemia and hypoalbuminemia.

**Keywords:** Hemodialysis, Non-Tunneled, Hemodialysis catheter, CABSI

### INTRODUCTION

The preferred modality of vascular access for hemodialysis is an arteriovenous fistula but Non Tunneled Hemodialysis catheters (NTHCs) remain the preferred vascular access for hemodialysis (HD) initiation in developing countries<sup>1-3</sup>. Central-venous-catheter-associated bloodstream infection (CABSI) is an important cause of hospital-acquired infection associated with morbidity, mortality, and cost. Consequences depend on associated organisms, underlying pre-morbid conditions, timeliness, catheter care and appropriateness of the treatment/interventions received.

**Aim:** To study the incidence and microbiological spectrum of NTHC-associated bloodstream infections (CABSIs) at a tertiary care center.

**Methods:** It was a prospective cohort study done over a period of one year. After approval by the Institutional Review Board; Ethics Committee and obtaining written informed consent, all adult ( $\geq 18$  years) hemodialysis patients who underwent NTHC insertion were included in this study.

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\*Resident, \*\*Senior Professor, \*\*\*, Assistant Professor, \*\*\*\*Senior Resident  
Department of Nephrology, SMS Medical College and Hospital, Jaipur, Rajasthan

#### Corresponding Author:

Dr Shivi, Senior Resident,

Department of Anaesthesia, SMS Medical College and Hospital, Jaipur, Rajasthan, India, 302004

Email id: [shashank.bhrdwaj@gmail.com](mailto:shashank.bhrdwaj@gmail.com)

Phone number: 8860144064

### Exclusion criteria:

1. Patient on immunosuppressing agents.
2. Patient with signs and symptoms of any systemic infections/sepsis of other cause.
3. Central venous catheters inserted for non-dialysis indications.

**Sample size:** To avoid selection bias all consecutive patients who met study protocol were approached for study participation.

**Data collection:** All patients presenting to the hemodialysis unit with clinical features of CABSIs were approached for inclusion in the study. After obtaining informed consent the demographic and clinical profiles were collected in a standard proforma. In all patients with clinical features suggestive of CABSIs (see definition below), paired blood cultures (10 mL each from the peripheral blood and venous catheter hub) were obtained under sterile conditions, inoculated in culture media and immediately transported to the microbiology laboratory. Peripheral blood cultures were obtained from peripheral vein. Non tunneled hemodialysis catheter were removed and its tip was also sent for culture. Plasma samples for total and differential white cell count were sent simultaneously. Catheter salvage was not attempted.

### Definitions

We followed the Centres for Disease Control and Prevention (CDC) guidelines<sup>4</sup> for diagnosis of central line-associated bloodstream infections. CABSIs were defined as bacteremia associated with intravascular catheter with all of the following elements:

1. In the case of common commensals like coagulase-negative staphylococcus (CoNS), both catheter and peripheral blood cultures growing the same organism; in the case of all other organisms, at least one positive blood culture (catheter hub or peripheral blood or both);
2. Clinical manifestations of infection (one or more of the following: fever  $>38^{\circ}\text{C}$ , chills or hypotension);
3. No other apparent source for the bloodstream infection and
4. Catheter in use within 48 hours of the CABSIs.

Statistical analysis: Independent t-test and Mann-Whitney U test was used to compare means between two groups and one-way analysis of variance or

Kruskal-Wallis H test was used to compare means between more than two groups, as applicable. For categorical variables, the Chi-square test was used to compare proportions. A 5% level of significance was considered significant.

### RESULT

In the study period, 1073 patients underwent hemodialysis catheter insertion. Out of these, 172 patients, who met the inclusion criteria were enrolled for this study. One patient was discharged against medical advice and was thus excluded. Data from 171 patients was analyzed. Mean age of this population was 45.57 years with a standard deviation of 16.99. 57.9% of total patients were males and 42.1% were females.

Out of 171, 40 patients (23.3%) developed CABSIs. Mean age of the patients having CABSIs was  $44.250 \pm 17.12$  years, while in those not developing CABSIs was  $45.977 \pm 17.004$  years. No statistically significant difference was found in age distribution in the two groups. Out of all the male patients, 24.2% developed CABSIs, while 22.2% females developed CABSIs. On statistical analysis, no significant difference was found in gender distribution as well.

Most common organism isolated was Coagulase Positive Staphylococcus aureus (40%). Other organisms isolated were Pseudomonas aeruginosa (22.5%), E. coli (15%), Klebsiella pneumoniae (7.5%), Enterobacter aerogenes (5%), Acinetobacter (5%), Enterococcus (5%) and Coagulase Negative Staphylococcus (staph epidermidis) 2.5%.

On an average, the HD catheter remained in situ for  $25.92 \pm 5.32$  days. In patients who developed CABSIs; mean days of HD catheterization before developing CABSIs was  $25.23 \pm 5.14$  while, it was  $26.14 \pm 5.37$  days in those patients who did not have CABSIs. No statistical significance was found for duration of HD catheterization prior having CABSIs.

On an average,  $14.743 \pm 6.534$  days were spent in hospital. Hospital stay increased significantly in those patients who developed CABSIs, with a mean duration of  $19.00 \pm 7.09$  days ( $P < 0.001$ ).

75 out of 171 patients (43.9%) had history of diabetes mellitus (DM). Out of 40 patients who developed CABSIs, 27 had history of DM. Among 131 patients who did not develop CABSIs, 48 were diabetic. Statistical analysis showed a significant association of DM with CABSIs, with  $p = 0.001$ .

51.5% of the enrolled patients had history of hypertension (HTN). 28 out of 40 patients who had CABSİ had hypertension. 60 were those who had hypertension but they did not develop CABSİ. On statistical analysis a significant association was seen between HTN and CABSİ ( $p=0.007$ ).

52.1% of total patients were found to be anemic. Out of these 29 developed CABSİ, while 60 did not. A statistically significant association was seen between anemia and CABSİ.

Similarly, statistical significance was also seen between low albumin levels and occurrence of CABSİ. A total of 71 patients were hypoalbuminemic. Out of these 24 developed CABSİ while 47 did not. Statistical analysis showed a  $p$  value of 0.007 and odds ratio of 2.7.

Most common symptom was fever (49.7%). 28 out of 40 patients who developed CABSİ had fever as primary complaint, while 57 patients who did not have CABSİ developed fever. A  $p$ -value of 0.003 showed statistical significance.

Local signs of infection were also evaluated. Our data showed that local site tenderness was most common (42.7%) sign present. But, no statistical significance was seen with development of CABSİ ( $p=0.978$ ).

Local site inflammation and pus discharge both were found to be statistically significant,  $p$  value 0.007 and 0.003 respectively. 16/40 patients who developed CABSİ had local inflammation while 7 of such patients had pus discharge. In 131 patients who did not develop CABSİ, 25 patients had local inflammation while 5 had pus discharge.

Overall mortality in this population was 7% (12/171). Out of 40 patients who had CABSİ, 9 died (22.5%). Chi-square test showed a significant correlation of CABSİ with mortality ( $p < 0.001$ ).

## DISCUSSION

This study collected data of 171 patients who underwent hemodialysis by Non-Tunneled Hemodialysis Catheters (NTHC). Analysis showed that 23.3% of the studied population developed CABSİ. These findings are similar to other studies<sup>5,6</sup>. No statistical significant difference was found in age and gender distribution among patients developing CABSİ and those who did not have CABSİ. Similar results were found in a study of 169 patients done by Varun et al.<sup>7</sup>. Catheter associated bloodstream infection (CABSİ) is responsible for

increased morbidity; mortality, more so in end stage renal disease patients<sup>8,9</sup>. This was corroborated by the findings of the current study. Average days spent in hospital was significantly increased in the patients who developed CABSİ ( $P < 0.001$ ). Similarly, mortality also increased significantly in these patients. Out of 40 patients who developed CABSİ, 9 died (22.5%).

Hypertension in patients is found to be an independent risk factor to develop CABSİ<sup>9</sup>. Similar results were found in our study. 70% of patients who developed CABSİ were hypertensive. Diabetes, anemia and hypoalbuminemia were also found to be significantly associated with CABSİ.

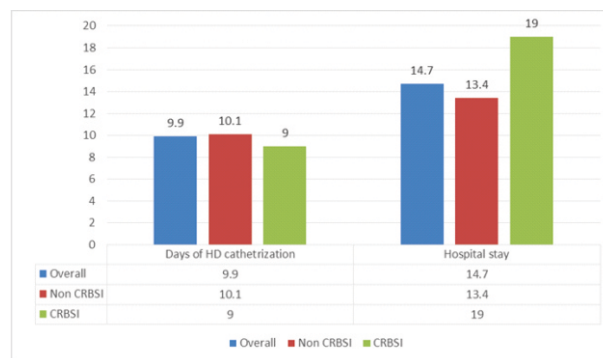
Majority of CABSİ (40%) in our study were because of Coagulase positive *Staphylococcus aureus*. These findings are consistent with several other studies<sup>6,10,11</sup>.

**Table 1: Risk assessment of CABSİ with several comorbidities**

	CABSİ	Non-CABSİ	p-value
Diabetes	27	48	0.001
Hypertension	28	60	0.007
Anemia	29	60	0.001
Hypoalbuminemia	24	47	0.007

In our study, on an average, the HD catheter remained in situ for  $25.92 \pm 5.32$  days. In patients who developed CRBSİ; mean days of HD catheterization before developing CRBSİ was  $25.23 \pm 5.14$  while, it was  $26.14 \pm 5.37$  days in those patients who did not have CRBSİ. No statistical significance was found for duration of HD catheterization prior having CRBSİ. Kairaitis et al<sup>10</sup> and Almiralli et al<sup>5</sup> in their respective studies also found that the risk of developing CABSİ at any point in time is constant.

## CONCLUSION



**Figure 1: Depiction of effect of CABSİ on duration of hospital stay.**

Non Tunneled Hemodialysis catheters (NTHC) associated CABSIs occur at a high rate with significant morbidity and mortality; especially in diabetics, hypertensive and those with anemia and hypoalbuminemia. Emphasis should be placed on early fistula creation in the course of chronic kidney disease, to avoid use of dialysis catheters. If absolutely required, tunneled catheters to be preferred. If NTHC are used should be placed for minimum required time, with meticulous aseptic and hygiene practices.

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# Guidelines for setting up of a Molecular Laboratory

Nidhi Sharma\*, Sandhya Gulati\*\*

### ABSTRACT

Molecular laboratories are now an essential part of diagnostics. The combination of sensitivity, specificity and speed has made molecular techniques appealing methods for the diagnosis of many diseases. While the requirements for setting up a molecular lab may vary depending on the spectrum of the tests that will be performed, there are several basic criteria that need to be fulfilled for standardization. Adequate space, appropriate equipment and qualified personnel are required to establish a molecular pathology laboratory. It is essential to take steps to prevent contamination. In this review, the criteria required to establish an optimal molecular pathology laboratory will be reviewed.

### INTRODUCTION

Molecular diagnostics is a technique used to analyse biological markers in the genome and proteome by applying molecular biology to medical testing. It is the detection and/or analysis of bio-molecules (DNA, RNA and Protein) for diagnostic and therapeutic purposes. The molecular laboratory is increasingly becoming an integral part of all laboratories. This is not only because of the advent of targeted therapies and personalized medicine in oncology but also as a gold standard diagnostic test in various benign conditions. The technologies that constitute molecular diagnostics include first-generation amplification, next-generation DNA sequencing, deoxyribonucleic acid (DNA) probes, fluorescence in situ hybridization (FISH), second-generation biochips next generation signal detection, biosensors, and molecular label<sup>1,2</sup>.

Interestingly, the establishment of molecular laboratory methods, the choice of the technique, the sample workflow, the quality control and the genomic alterations to be detected, are always left to the discretion

of the laboratory. The purpose of this article is to describe the different steps of the settlement of a molecular genetic platform in a pathology laboratory.

### Take the first step, howsoever small

As a first phase, molecular laboratories are established to develop a selection of genes to evaluate a few genomic alterations. In a second phase, the number of studied genes is increased. The challenge to laboratories is to meet the increasing need, using reliable methods and processes to ensure that patients receive a timely and accurate report on which their treatment will be based. On the successful launch and utilization of real-time PCR technologies, lab can further go to Phase 2 analytical instruments like sanger (dye termination) sequencing and/or NGS.

### Scope of Applications of Molecular Diagnostics

From the detection, quantitation, genotyping of various infectious agents (Viruses, bacteria, fungi, and parasites) to detection of defective genes and variations in the genome, the scope of molecular genetics is tremendous. Congenital genetic disorders, diagnosis of existing disease or predisposition to any, malignancies, gene defects, expression profiles related to cancer, pharmacogenetics (identification of slow, average, fast metabolizers, non-responders) to optimize drug therapy, paternity, forensic medicine and transplantation, the uses of molecular techniques are far from imagination.

### Three basic requirements for setting up a molecular lab

To begin with, the three broad requirements for a molecular lab are:

- 1) Adequate and well organized space
- 2) Essential lab equipments

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\* Associate Professor, \*\* Senior Professor

Department of Pathology, SMS Medical College, Jaipur

**Corresponding Author**

Dr. Sandhya Gulati

Sr. Professor, Department of Pathology, SMS Medical College, Jaipur

Email- [sandygulati60@gmail.com](mailto:sandygulati60@gmail.com)

Mobile. No. 9829010184

3) Well trained and competent staff including doctors, lab technicians, post docs.

These three requirements will be discussed in detail.

### Workflow for molecular diagnostics:1) Organizing the space

The various steps of molecular diagnostic lab include: 1) sample collection 2) DNA/RNA extraction 3) DNA amplification 4) real time analysis 5) gel analysis. The entire workflow is divided into two main phases a) pre-analytical and b) analytical (figure 1).

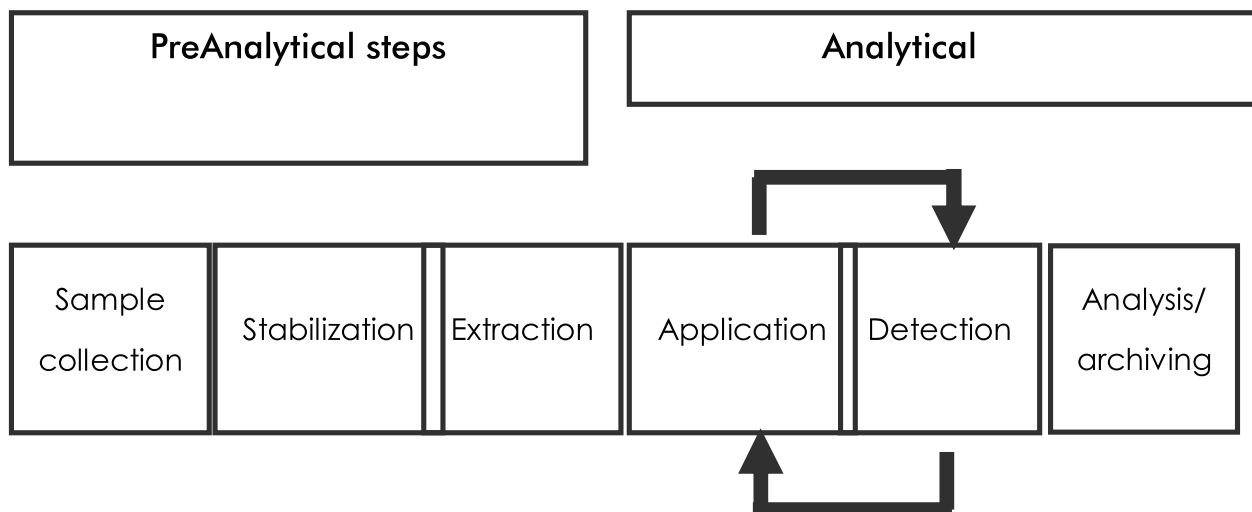


Figure 1: Workflow of a molecular lab

### Preanalytical considerations

An important step in molecular testing is a careful consideration of the way in which specimens are obtained and reach the laboratory<sup>3</sup>. Sample receipt and handling follow standard operating procedures. DNA and RNA extraction need to be standardised and should be checked for quality and quantity of output on a regular basis. Internal quality control, regular internal audit of the whole testing process, laboratory accreditation, and continual participation in external quality assessment schemes are prerequisites for delivery of a reliable service. A molecular pathology report should accurately convey the information the clinician needs to treat the patient with sufficient information to allow for correct interpretation of the result.

### Ideal physical conditions

The ideal is a 5-room set up, though in case there are space considerations it can be done in a 4/3-room set up also (Figure 2). The three minimum areas are:

**Area 1 – Reagent preparation:** it is the room where reagent stocks are prepared and then divided into a certain number of small usable parts (aliquoted), and the reaction mixes are prepared. This room should be free of any biological materials such as DNA/RNA extracts, PCR

products.

**Area 2 – Specimen/control preparation, PCR set-up:** It is where the nucleic acid isolation is performed and the isolated samples are added to the PCR reaction mixes. This room is also called a “low copy” room, as the number of copies has not yet been amplified by PCR. Ideally, it is recommended to perform the steps of nucleic acid isolation and addition of isolated samples to the PCR reaction mixes in separate rooms. Preparation of the PCR reactions in a laminar flow biosafety cabinet ensures that the area remains clean.

**Area 3 – Amplification/product detection, plasmid preparation:** It is where PCR devices are located and the amplification steps are performed.

Two of these rooms are considered clean rooms in that all tasks and duties before in vitro amplification are performed in these laboratories<sup>4</sup>. These rooms are called preamplification rooms. The third room is considered a dirty room because it is dedicated for in vitro amplification and analysis of the amplified material. This room is called a postamplification room.

The preamplification laboratories should be under positive air pressure, which impedes the entrance of any airborne contaminant into the preamplification

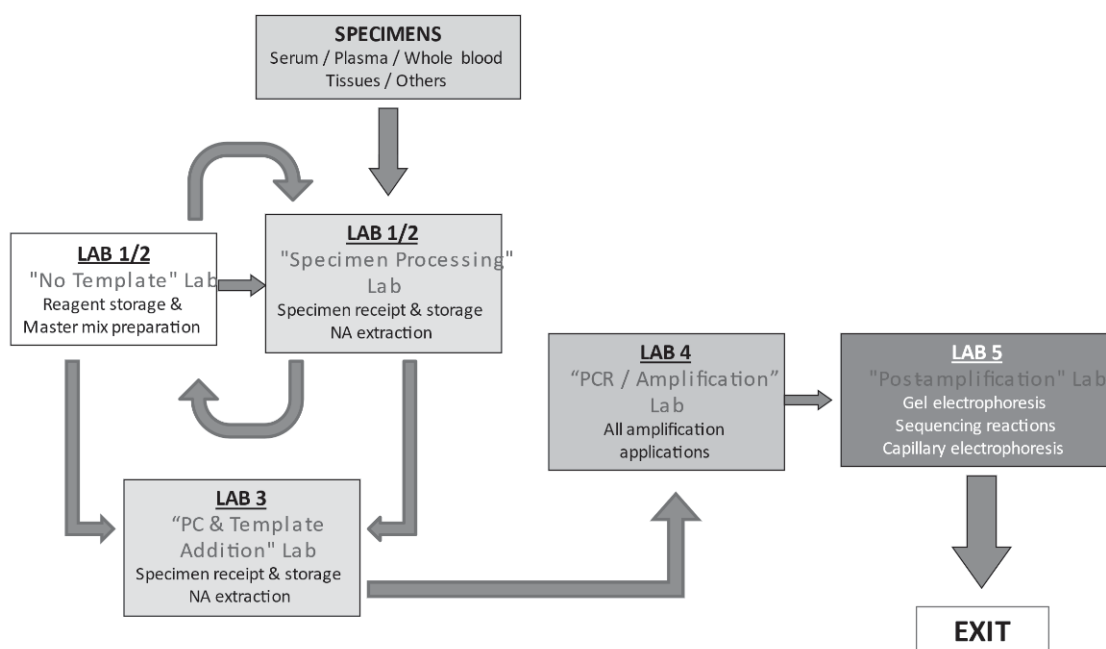
laboratory when the door is opened. In addition, the postamplification laboratory should be under negative air pressure to impede anything from coming out of this laboratory and contaminating the surrounding environment.

### Ideal workflow

There should be mechanical barriers between these areas to prevent contamination. The workflow should always be **unidirectional** for all personnel,

including cleaning personnel, and specimens. Remove PPE before leaving one area. Avoid or limit reverse direction. Reusable supplies in the reverse direction need to be bleached. Reagents used for amplification should not be exposed to other areas. Size of each area should consider space for equipment and bench space needed for preparation. Other laboratory design considerations include temperature and humidity requirements, exhaust ventilation, water quality, electric outlets, back-up power system and ergonomic assessment.

## Ideal Lab Workflow for PCR and qPCR applications Option A 5 Room Set-up



**Figure 2: Ideal workflow for molecular lab**

### 2) Equipment required for a molecular lab

PCR labs typically require a variety of equipment, such as centrifuges, vortex mixers, pipettes, fridges and freezers, thermal cyclers and analysis

instruments (e.g., electrophoresis systems). Depending on the size of lab and applications, the number of equipment may vary. Since aerosols are always a concern, a laminar flow hood or biosafety cabinet is must. The main equipment required in each area is described in table 1.

**Table 1: Ideal lab set-up for PCR and qPCR applications**

Area	Equipment
Common Instrument Area	Ice Flaker
	Autoclave – Front loading / manual / digital
	pH Meter – Digital / manual
	Plate Shaker
Specimen Processing	Biosafety cabinet
	Refrigerated Centrifuge
	DNA/RNA extractor
	Mini Spin and vortex
	Dry Heating Block
	Spectrophotometer (Nano Drop / Qubit)
	Dedicated pipette Set
	Chillers (4° C) and Refrigeration System (-20° C) and (-80)
Reagent Preparation	PCR Hood / workstation / clean hood
	Mini Spin and vortex
	Dedicated pipette Set
	Refrigeration System (-20° C)
Amplification / PCR	Thermal Cycler
	Real-Time PCR instrument
	DNA Sequencer
Post - Amplification	Microwave oven, Gel Electrophoresis System with Power Packs and Casting Units
	Gel Documentation
	Automated Elisa System

### 3) Well trained staff

Machines cannot run on their own and can definitely not replace human mind. Experienced and trained doctors, researchers and technicians run the machines. Expertise in the molecular biology field along with the dedication of the staff towards running the lab is one of the most essential requisites for successful running of a lab. All that glitters are not gold; the shining molecular lab often has volumes to tell about the hard work of the involved staff. It is essential for staff to remain updated about the latest techniques of molecular biology.

#### Quality Management Components

Various aspects of the molecular lab require quality management. These include proper organization, trained personnel, maintenance of documents and records, environment and safety and use of referral laboratories whenever needed<sup>5,6</sup>.

Laboratory Practices which ensure quality are use of positive displacement pipettes and disposable filtered pipette tips, avoiding production of aerosols when

pipetting, use of sterilized single-use plasticware, use of hairnet and dedicated safety glasses, disposable lab coat/gown, and shoe covers. Use of nuclease free or autoclaved water avoiding multiple freeze thaws which can cause degradation. Always include a blank (no template) control to check for contamination.

#### Contamination

Introduction of unwanted nucleic acids into specimen which hampers the sensitivity of PCR techniques makes them vulnerable to contamination. Repeated amplification of the same target sequence leads to accumulation of amplification products in the laboratory environment. Build-up of aerosolized amplification products will contaminate laboratory reagents, equipment, and ventilation systems.

The potential sources of contamination include:

- 1) Cross contamination between specimens
- 2) Amplification product contamination
- 3) Laboratory surfaces

- 4) Ventilation ducts
- 5) Reagents/supplies
- 6) Hair, skin, saliva, and clothes of lab personnel.

### Decontamination Approaches

Always chose sterile products from manufacturers that can certify that their tips and consumables are free of any of these potential contaminants<sup>7</sup>. Clean the work area & equipment routinely, clean the PCR workstation at the start and end of each work day/run (UV light, 70% ethanol, fresh 10% sodium hypochlorite). Clean the exterior and interior parts of the pipette. Clean the equipment, doorknobs, handle of freezers. Despite being more expensive than normal pipette tips, using filter tips for your PCR set-up will avoid aerosols entering and contaminating your pipette, and avoid aerosols that might already be present in your pipette contaminating your master mix or samples. Adding the sample last is also recommended. Aliquoting reagents into smaller containers will increase their shelf life and prevent them from going through too many freeze/thaw rounds<sup>8</sup>.

### When is a Validation/Verification Study Required?

Whenever a new testing system or a new analyte is introduced in the lab or an analyte previously measured/detected is now measured on an alternate system, a validate is required.

### Types of Controls

The types of controls that are used are:

1) Positive amplification controls: it checks whether the experiment is capable of recovering and amplifying the target. It detects failure of DNA extraction or PCR amplification, reagent or equipment issues, Integrity of DNA sample, presence of inhibitory substance. Negative control checks for contamination of PCR experiment with foreign DNA. This includes 2) Negative Control: checks if there is any contamination of the experiment with foreign DNA. 3) No template control (extraction blank): checks if PCR reagents are contaminated. 4) Internal Control. 5) External Control

### CONCLUSION

It is necessary to carefully design the molecular diagnostics laboratory to ensure sufficient and adequate space for personnel and equipment and also to minimize the potential problem of specimen contamination. The ideal lab set up is sometimes far from real and the labs may

have to adjust in terms of space, staff and budget. It's best to just get started and evolve!

**Future directions:** The use of molecular laboratory in molecular diagnostics, such as pre-implantation diagnostics or predictive genetic testing, still has technical problems as well as a novel and unclear, social, ethical, and legal implications. The scope of molecular laboratory in molecular medicine could be expanded well beyond current nucleic acid testing which plays an important role in the practice of medicine, public health, pharmaceutical industry, forensics, and biological warfare, and drug discovery. The molecular laboratory marketplace offers a growth opportunity given the interest in utilizing molecular tools to precisely target therapeutics.

### Take Home Messages

- ✓ The molecular laboratory has become a growing part of the clinical laboratory.
- ✓ Separate laboratory spaces are needed for reagent preparation, sample preparation, amplification and detection.
- ✓ Unidirectional workflow is a must.
- ✓ Precautions and special laboratory practices must be made to minimize the risk of contamination and decontamination steps should be done.
- ✓ A quality control plan to monitor the quality of testing process and detect errors should be in place.
- ✓ Continuous quality improvement is essential.
- ✓ Accreditation is going to be vital and unavoidable.

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## DRUG UPDATE

### Gefapixant

Monica Jain\*, Jaya Dadhich\*\*, Kopal Sharma\*\*\*

#### ABSTRACT

Chronic cough lasting for more than 8 weeks can be due to some underlying pathological conditions or can be unexplained. Chronic cough leads to decrease in quality of life. After several investigations and researches it has been found that it can be benefitted by some agents working on cough reflex pathway. One of such agents is, Gefapixant which is an investigational selective P2X<sub>3</sub> receptor antagonist. This drug update will describe all the clinical trial data, benefits and potential uses of this novel agent.

**Keywords;** Chronic cough, Gefapixant, P2X<sub>3</sub> antagonist

#### Article

Chronic cough affects approximately 5–10% of the global population. Chronic cough can be complicated by incontinence, cough syncope, and dysphonia, leading to social embarrassment and subsequent social isolation and depression or anxiety leading to poor quality of life.

Chronic cough can be of two types refractory or unexplained. In some people it can be associated with a treatable underlying cause such as allergic rhinitis, upper-airway cough syndrome, asthma, non-asthmatic eosinophilic bronchitis or gastro-oesophageal reflux disease. Many of these individuals might respond to treatments targeting the primary cause. This is called as refractory chronic cough, while unexplained is the one which is not getting treated and is idiopathic. Currently, no therapies are approved for these conditions, and for treatment of such cases, clinicians often focus on off-label treatments such as morphine, amitriptyline, gabapentin, pregabalin, speech therapy etc. Thus to cover these unmet needs for safe and effective medication, one new development is coming in picture elaborating cough as

neuropathic disorder and targetting newly involved receptors P2X<sub>3</sub> in pathophysiology of cough<sup>1</sup>.

P2X<sub>3</sub> receptors are ATP ion-gated channels located on primary afferent neurons. ATP released from damaged or inflamed tissues in the airways acts on P2X<sub>3</sub> receptors of primary afferent neurons, trigger depolarization and action potentials which is then transmitted centrally and results in cough. There are strong preclinical and clinical evidence supporting the role of P2X<sub>3</sub> receptors in hypersensitization of the cough reflex. Following diagram explain cough reflex pathways and role of different receptors in mediation of cough reflex<sup>2,3</sup>. (Figure 1 & 2)

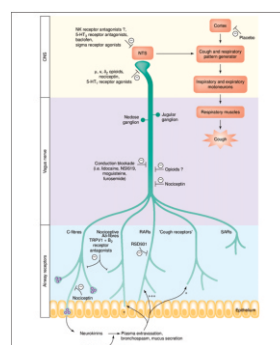


Figure-1

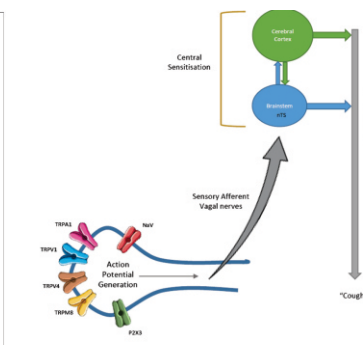


Figure-2

Preclinical studies had shown that P2X<sub>3</sub> receptors, which are present on airway vagal afferent nerves, are countable for hypersensitisation of sensory neurons and mediate the cough reflex, which leads to chronic cough. Preclinical studies were performed to investigate the efficacy of AF-219, to reduce cough frequency in patients with refractory chronic cough and they gave satisfactory results. Gefapixant is a first-in-class, non-narcotic, selective antagonist of the P2X<sub>3</sub> receptor. This drug has shown efficacy and was also very well tolerated in phase 2 and phase 3 clinical trials in patients with refractory chronic cough<sup>4,5</sup>.

\*Senior Professor, Department of Pharmacology, SMS MC, Jaipur.

\*\*Sr. Demonstrator, Department of Pharmacology, SMS MC, Jaipur.

\*\*\*Assistant Professor, Department of Pharmacology, SMS MC, Jaipur.

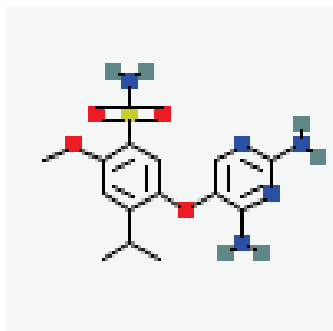
#### Corresponding Author

Dr. Jaya Dadhich, Sr. Demonstrator, Department of Pharmacology, SMS Medical College, Jaipur

Email- [Jayadadheech.doc@gmail.com](mailto:Jayadadheech.doc@gmail.com), Contact No. 9461602550

Chemistry of Gefapixant<sup>6</sup>

- Chemical structure of Gefapixant



- Molecular formula-  $C_{14}H_{19}N_5O_4S$
- MW: 353.4g/mol
- IUPACName: 5-(2, 4-diaminopyrimidin-5 - y l) o x y - 2 - m e t h o x y - 4 - p r o p a n - 2 - y l b e n z e n e s u l f o n a m i d e<sup>6</sup>

In one of the double-blind, placebo-controlled, two-period, crossover study, a computer-generated sequence was used to randomly divide patients with refractory chronic cough in two groups receiving AF-219 (Gefapixant), 600 mg twice a day and placebo (1:1), and then, after a 2 week washout, assigned patients received the other treatment. Patients, health-care providers, and investigators were masked to sequence assignment. Daytime cough frequency (primary endpoint) at baseline and after 2 weeks of treatment using 24 h ambulatory

cough recordings was noted. This study showed that Targeting purinergic receptor P2X3 with Gefapixant at a dose of 50 mg twice daily significantly reduced cough frequency in patients with refractory chronic cough or unexplained chronic cough after 12 weeks of treatment compared with placebo. Further development of gefapixant is warranted for the treatment of chronic cough<sup>7</sup>.

In another phase 2b, randomised, double-blind, placebo-controlled study in USA and UK again this compound was tested. This was a 12 week study in patients with refractory chronic cough or unexplained chronic cough in age group of 18-80 years who were recruited from 44 primarily outpatient respiratory departments. Recruited patients had refractory or unexplained chronic cough for 1 year or longer, no radiographic chest abnormality, and 40 mm or more on a 100-mm cough severity visual analogue scale at enrolment. According to a computer generated system patients were randomly assigned to receive placebo or oral Gefapixant (in one of three doses of 7.5 mg, 20 mg, or 50 mg twice daily) every day, for 84 days. Changes in cough frequency was assessed after 12 weeks in the full analysis set, along with this adverse events were also monitored to evaluate safety. Dose of 50 mg was found very effective in reducing cough frequency and also was not having a lot of adverse events<sup>8</sup>.

	COUGH-1 (Week 12)			COUGH-2 (Week 24)		
	Placebo	Gefapixant 15 mg	Gefapixant 45 mg	Placebo	Gefapixant 15 mg	Gefapixant 45 mg
<b>Efficacy</b>						
N Included in Analysis	222	227	217	419	415	409
Baseline Geometric Mean 24-Hr Cough Frequency (coughs/hr)	22.83	19.86	18.24	19.48	19.35	18.55
Geometric Mean 24-Hr Cough Frequency at Primary Timepoint	10.33	9.66	7.05	8.34	8.10	6.83
Estimated Relative Reduction (%) (95% CI) vs Placebo*	--	1.58 (-16.12, 23.01)	-18.45 (-32.92, -0.86) p=0.041	--	-1.14 (-14.27, 14.02)	-14.64 (-26.07, -1.43) p=0.031
<b>Safety</b>						
N included in summary (Safety)	243	244	243	433	441	440
% Overall AEs	53%	56%	75%	73%	79%	87%
% Serious AEs	2%	3%	3%	4%	3%	3%
% Taste-Related AE	3%	11%	58%	8%	20%	69%
*Estimated relative reduction (%) vs placebo was estimated by $100 * (\exp(\text{diff}) - 1)$ , where diff was the difference provided by the analysis of the log transformed variable.						

Two phase 3 trials of Gefapixant which were named as COUGH-1 (NCT03449134) and COUGH-2 (NCT03449147). These were double-blind, randomized placebo-controlled trials for refractory or unexplained chronic cough (RCC/UCC). This table shows the results of these two trials<sup>8</sup>.

Treatment with Gefapixant 45 mg BID resulted in significant reduction in 24h cough frequency in participants with RCC/UCC. Serious AEs were infrequent and AEs with Gefapixant 45 mg were most commonly related to taste<sup>9</sup>.

In conclusion it can be said that Gefapixant seems to be an efficacious treatment option for chronic cough with an acceptable safety profile and no serious treatment-related adverse events. Still some comparative studies with other available options and extensive research on adverse effect profile is required.

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## CASE REPORT

### Endometrial osseous metaplasia: Clinicopathological study.

Mukesh Mittal\*, Pankaj Kumar Nitharwal\*\*, Mukesh Bijarniya\*\*\*, Batti Lal Meena\*\*\*\*, Vaishali Singh\*\*\*

#### ABSTRACT

Osseous metaplasia of the endometrium is an uncommon disorder associated with the presence of bone in the uterine endometrium. Most of the cases present with secondary infertility after an abortion. We report a case of a 21-year-old woman who presented with intermittent bleeding per vagina due to osseous metaplasia in the endometrial cavity. Multiple theories have been proposed and the most accepted theory is metaplasia of the stromal cells into osteoblastic cells that produce the bone. Removal of these bony parts from endometrium leads to normal menstrual cycle.

#### INTRODUCTION

Osseous metaplasia of the endometrium is a rare pathology in which abnormal bone formation occurs in the endometrium. In most of the cases, ossification was followed by an abortion and patient presented with infertility. Patient may also present with symptoms such as menstrual abnormalities, dysmenorrhea, pelvic pain and abnormal vaginal discharge. The bone in the endometrial lining acts as an intrauterine device and it can be a cause of infertility. Hysteroscopic removal of the bony fragments usually results in resolution of most symptoms and return of fertility.

#### CASE PRESENTATION

A 23-year-old female patient presented to gynecology OPD department with the history of intermittent bleeding per vagina, dysmenorrhea and lower abdominal pain since 2 months with H/O normal vaginal delivery before 8 months.

Trans-abdominal USG revealed an echogenic area in the uterine cavity measuring 19 x 6 mm with distal acoustic shadow without any vascularity suggestive of calcification (figure 1).

On the basis of the imaging findings and clinical history, the presumptive diagnosis was endometrial osseous metaplasia which was confirmed by histopathology.

The hematoxylin and eosin stained paraffin sections showed trabeculae intermingled with endometrial tissue. Retained products of conception, inflammatory reaction and necrosis were absent. No evidence of granuloma was seen (figure 2).

The osseous fragments were removed with the help of graspers of a rigid hysteroscope. Since the fragments were not deeply embedded, hysteroscopic resection was not necessary in this patient.

Postoperative sonography revealed a normal endometrial lining and on histopathology diagnosis of osseous metaplasia of the endometrium was confirmed.

#### DISCUSSION

Ossification of the endometrium is a rare clinical entity. It has many synonyms such as endometrial ossification, ectopic intrauterine bone, and heterotopic intrauterine bone. In most of the reported cases, the osseous changes in the endometrium were followed by a previous history of abortion<sup>1</sup>. Majority of the patients are in the reproductive age group with history of first trimester abortion either therapeutic or spontaneous and have normal vaginal delivery. The time interval between the antecedent abortion /vaginal delivery and discovery of endometrial ossification varies from 8 weeks to years in reproductive age group<sup>2</sup>.

Common clinical presentations are menstrual irregularities, pelvic pain, dysmenorrhea, vaginal discharge, and secondary infertility. Endometrial ossification is described as an endogenous non-neoplastic pathological condition without tissue reaction in the endometrial tissue and the endometrium showed normal

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\*Professor, Department of Radiodiagnosis, SMS Medical College, Jaipur,

\*\*Assistant Professor, Department of Radiodiagnosis, SMS Medical College, Jaipur

\*\*\*Junior Resident, Department of Radiodiagnosis, SMS Medical College, Jaipur

\*\*\*\*Assistant Professor, Department of Pathology, SMS Medical College, Jaipur

#### Corresponding Author:

Dr. Pankaj Kumar Nitharwal, Assistant Professor, Department of Radiodiagnosis, SMS Medical College, Jaipur

Mobile: 8447445938

Mail ID: [pankaj6468@gmail.com](mailto:pankaj6468@gmail.com)

regular cyclical changes with presence of osseous trabeculae<sup>2</sup>.

The pathogenesis of this rare condition is still disputed. The two most accepted mechanisms involve either the presence of chronic endometriosis with undifferentiated mesenchymal cells inducing the endometrial stromal cells transformation into osteoblast, or miscarriage with dystrophic ossification of the residual product of conception<sup>2,3</sup>.

Infertility is caused by the action of these osseous trabeculae similar to the action of an intrauterine contraceptive device (IUCD). Infertility and endometrial calcification and subsequent ossification can be caused by endometrial tuberculosis in India<sup>4</sup>.

Previously treatment of this ossification of the endometrium was a series of dilatation and curettage to remove the bony trabeculae from the endometrium. Vigorous single curettage can further lead to formation of synechiae<sup>2</sup>. Ultrasonic guided hysteroscopic removal of these osseous trabeculae helps proper visualizations and complete removal of the bony spicules that may be embedded in the myometrium<sup>1</sup>.

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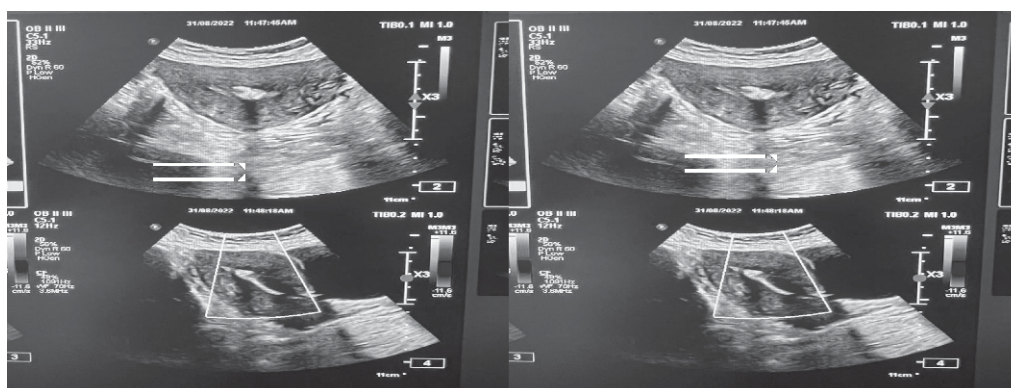


Figure 1 Trans-abdominal ultrasound showing linear calcifications in the uterine cavity

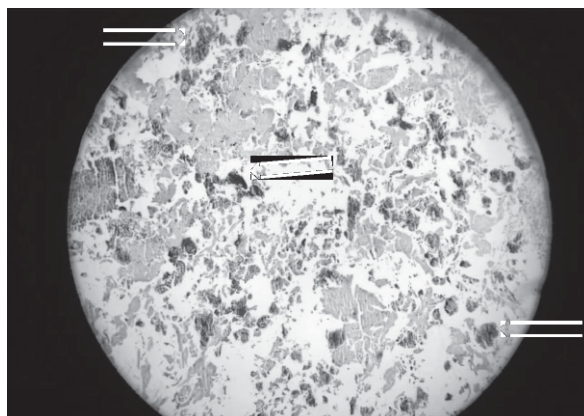


Figure 2. Photomicrography with low and medium magnification showing osseous trabecula

## CASE REPORT

# Osteofibrous Dysplasia Disguising as Chronic Osteomyelitis: A Diagnostic Dilemma

Mukesh Mittal\*, Isha Gupta\*\*, Batti Lal Meena\*\*\*, Pankaj Kumar Nitharwal\*\*\*\*, Harshita Sharma\*\*, Laxmi K Mallya\*\*

## ABSTRACT

Osteofibrous dysplasia is a rare benign fibro-osseous lesion with a strong predilection for involvement of tibia in the early childhood. Its biologic behaviour ranges from non-progressive to recurrent and more aggressive lesions however, most lesions become quiescent or even regress spontaneously as skeletal maturation is completed. We report a rare case of a 20 years old male patient admitted with a history of swelling in the left hand with complaints of pain and local signs of inflammation. Radiography and magnetic resonance imaging were done and indicated the involvement of the head and distal shaft of the second metacarpal with bone marrow edema, destruction of cortex and surrounding soft tissue edema. All the clinical features and radiological imaging were in favour of chronic osteomyelitis. Patient underwent surgery and biopsy indicated presence of osteofibrous dysplasia highlighting the importance of considering this differential diagnosis in such presentations.

## INTRODUCTION

Osteofibrous dysplasia is a rare benign fibro-osseous lesion with a strong predilection for involvement of tibia in the early childhood. Histopathologically it is a benign fibro-osseous lesion composed of fibrous tissue with woven bone formation. It is most commonly found in the tibia and fibula, although a case with ulnar involvement has been reported<sup>1</sup>.

Its biologic behaviour ranges from non-progressive to recurrent and more aggressive lesions however, most lesions become quiescent or even regress spontaneously as skeletal maturation is completed. Radiographic findings include eccentric, fairly well-marginated osteolytic lesion with a sclerotic border in the

anterior cortex of the tibial diaphysis and can show longitudinal spread to metaphysis, cortical expansion, intramedullary extension and anterior bowing deformity.

Chronic osteomyelitis of the hand is uncommon and usually due to *Staphylococcus aureus* and affects a single bone. Involvement of the metacarpals occurs in only 3% of cases<sup>2</sup>.

Differential diagnosis of osteolytic lesions of bone ranges from tumors to non-tumorous pathologies like infective lesions or metabolic problems<sup>3</sup>. Imaging can help narrow down the differentials however, biopsy is essential in most cases to identify the pathology, permitting a specific therapeutic approach. We report a rare case of osteofibrous dysplasia of metacarpal whose clinical and radiological findings were in favour of osteomyelitis which was turned out as osteofibrous dysplasia on histopathology.

## CASE REPORT

A 20-year-old male presented with a swelling over the dorsum of left hand for four months with an increase in the size of swelling with development of pain over the past 15 days. He complained of feeling feverish on and off over the past 15 days but no definitive documentation of fever was present. On physical examination swelling and tenderness was present proximal to the left second metacarpophalangeal joint with evidence of local inflammation.

On X-ray examination, an osteolytic lesion with narrow zone of transition (Figure 1) was noted in the metaphysis of the left second metacarpal proximal to its head with suspicious break in cortex. Smooth periosteal reaction was noted. No associated expansion of bone or soft tissue component was seen. Other metacarpals were normal.

\*Professor, Department of Radio diagnosis, SMS Medical College, Jaipur

\*\*Junior Resident, Department of Radio diagnosis, SMS Medical College, Jaipur

\*\*\*Assistant Professor, Department of Pathology, SMS Medical College, Jaipur

\*\*\*\*Assistant Professor, Department of Radio diagnosis, SMS Medical College, Jaipur

## Corresponding Author:

Dr. Pankaj Kumar Nitharwal

Assistant Professor, Department of Radio diagnosis, SMS Medical College, Jaipur

Mobile: 8447445938 E-mail: pankaj6468@gmail.com

On MRI examination, the lesion corresponded to altered marrow signal intensity appearing hypointense compared to surrounding marrow on T1 and T2 (Figure 2) and hyperintense on PD-FS image (Figure 3). Bone marrow edema was noted extending to the head and shaft of the metacarpal. Thinning of cortex with focal breach was noted near anteromedial aspect of distal metaphysis of the second metacarpal with surrounding circumferential periosteal reaction and soft tissue edema. The distal articular surface of the metacarpal appeared normal and intact. The lesion was categorized as osteomyelitis according to the clinical presentation and radiological findings.

CT guided biopsy of the lesion was performed which on histopathological examination (Figure 4) revealed irregular fragments of woven bone and lamellar bone lined by osteoblasts with fibrous tissue component consisting of spindle cells and infiltrating collagen, with myxoid stroma. These findings were suggestive of presence of osteofibrous dysplasia lesion in the second metacarpal with an associated pathological fracture.

## **DISCUSSION**

Osteolytic lesions of bone can have a wide range of differentials from infective to metabolic to neoplastic lesions. Chronic osteomyelitis is one of the differentials of an osteolytic lesion involving the metaphysis of metacarpal. More than two-thirds of patients with chronic osteomyelitis of the hand have involvement of a single bone<sup>10</sup>, and young males are predominantly affected. It usually presents as an inflammatory swelling of the hand with functional incapacitation. A fever is uncommon in chronic cases and occurs chiefly in patients with an acute onset or a concomitant infection at another site<sup>3</sup>. Plain radiograph should be obtained routinely; they show bony destruction and a periosteal reaction, a combination highly suggestive of chronic osteomyelitis<sup>9, 14</sup>. Computed tomography is useful for detecting early evidence of bone destruction and periosteal reaction; however MRI is more sensitive and provides an accurate evaluation of the lesions, the bone marrow changes and changes in the surrounding tissues. The abnormal bone generates low signal on T1 images and high signal on T2 images<sup>3</sup>.

Osteofibrous dysplasia, also referred to as

ossifying fibroma of long bones, is a benign fibro-osseous lesion composed of fibrous tissue with woven bone formation. The lesions mostly occur in the tibia and fibula, although a case with ulnar involvement has been reported<sup>6</sup>. The features that differentiate it from fibrous dysplasia include the characteristic zonal phenomenon and osteoblastic rimming of bony trabeculae which are absent in fibrous dysplasia.

Earlier Osteofibrous dysplasia was referred to as ossifying fibroma of long bones. Although ossifying fibroma and osteofibrous dysplasia of the jaw have few common histological characteristics like the presence of cytokeratin-positive cells in the ossifying fibroma in the long bones and development of psammomatous calcification in the ossifying fibroma of the jaw are exclusive histopathological features that distinguish the two as separate diseases<sup>4</sup>.

Osteofibrous dysplasia usually appears as a loculated osteolytic lesion circumscribed by a sclerotic border involving the anterior diaphyseal cortex of the tibia or fibula with adjacent cortical expansion. Intramedullary encroachment and anterior bowing deformity are commonly associated as the lesion progresses<sup>1</sup>. The signal intensity of osteofibrous dysplasia was intermediate on T1-weighted images and intermediate to high on T2-weighted images<sup>1</sup>. Superimposed haemorrhagic or cystic, myxoid change and even cartilaginous differentiation can modify the signal intensity and contribute to heterogeneous signal intensity on T2-weighted images<sup>5</sup>. In the study by Jung JY et al, complete medullary involvement was observed in 33% of osteofibrous dysplasia cases and soft tissue edema was generally absent or mild without pathologic fracture<sup>1</sup>.

## **CONCLUSION**

Although imaging can help narrow down the differential diagnosis, often it is not enough and histopathology is needed to come to an accurate diagnosis. We report a rare case of osteofibrous dysplasia affecting the metacarpal. This rare case suggests that Osteofibrous dysplasia can exhibit diverse imaging features with lesions with complete intramedullary involvement or perilesional marrow edema, pathological fractures and involvement of uncommon locations like metacarpals.

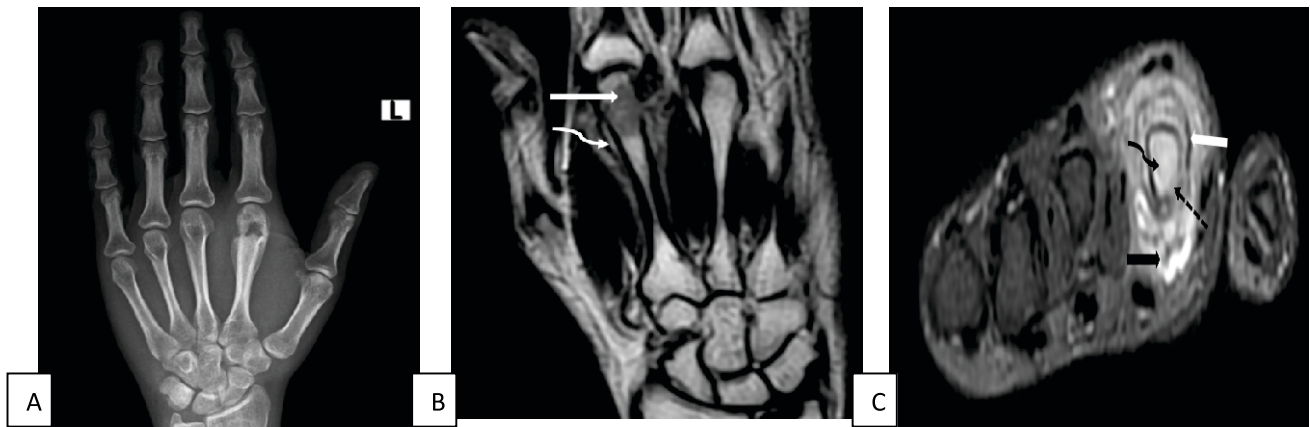


Figure 1: A - Radiograph of left hand (PA view) showing an osteolytic lesion with a narrow zone of transition, surrounding circumferential smooth periosteal reaction in distal metaphysis of second metacarpal. B - Coronal T2 W MRI image showing altered marrow signal intensity (white solid arrow) appearing hypointense compared to surrounding marrow in distal metaphysis of the second metacarpal with surrounding circumferential periosteal reaction (curved white arrow). C - Axial PD-FS MRI image showing marrow edema (curved black arrow) with discontinuity of cortex (dashed black arrow) near anteromedial aspect of distal metaphysis of the second metacarpal with surrounding circumferential periosteal reaction (white solid arrow) and soft tissue edema (solid black arrow).

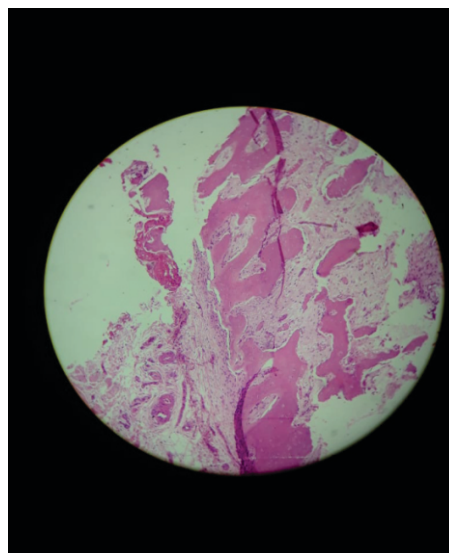


Figure 2: Irregular fragments of woven bone and lamellar bone lined by osteoblasts. Fibrous tissue component was also noted consisting of spindle cells and infiltrating collagen, with myxoid stroma. Mitosis were rare.

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## CASE REPORT

# Management of a Symblepharon Case by Auto Conjunctival Graft: A Clinical Case Report

Raghunandan Khandelwal\*, Archana Garg\*\*, Rakesh Porwal\*\*\*

## ABSTRACT

**Background:** A twelve years old male child presented in our OPD with symblepharon in the inferior cul de sac of the right eye with H/O lime chemical injury 23 days back. The patient was complaining about eye mass, redness, watering, foreign body sensation, and cosmetic consideration in the right eye. His visual acuity was 6/18 in the right eye. IOP in both eyes was 14.6 mm of Hg at the time of admission. The cornea was partially opaque near the symblepharon site and extra-ocular movements were partially restricted due to adhesion.

**Management:** symblepharonectomy has been performed in the inferior cul-de-sac of the right eye. The auto conjunctival graft was taken from the superior conjunctiva of the same eye and sutured to the symblepharonectomy site of the right eye. A conformer was placed to the right eye to prevent recurrent symblepharon.

**Discussion:** Next day evaluation showed an inferior cul-de-sac of the right eye was formed, and the conjunctiva was viable and ready to get an ocular prosthesis. Conjunctival graft and conformer placing can prevent recurrent symblepharon after symblepharonectomy. It may provide a cheap and simple way to form a barrier that prevents reattachment of the eyeball and eyelids.

**Keywords:** symblepharonectomy, auto conjunctival graft, conformer.

## INTRODUCTION

Symblepharon is a pathological condition where the bulbar and palpebral conjunctiva form an abnormal adhesion to one another<sup>1</sup>. Most cases of symblepharon are acquired. It may be caused by autoimmune diseases, mechanical, thermal, or chemical injury, and as a complication of conjunctival surgery<sup>2</sup>. Treatment of symblepharon by chemical injury is challenging as it may

be accompanied by tear film instability, tear reduction, and cicatricial entropion. In the later stage, there may be the development of irregular corneal surface, inflammation, conjunctivalization, corneal vascularization, and poor epithelial integrity<sup>3</sup> which may lead to the dysfunction of the ocular surface and may affect the treatment and prognosis of symblepharon. Surgical correction with lubricant application is the mainstay of treatment. Symblepharectomy invariably creates a defect in the conjunctiva. If left uncovered, might result in re-adhesion of the exposed surfaces. To cover the defects conjunctival<sup>4</sup>, the amniotic membrane, oral mucosa, and nasal mucosa are alternative tissues.

## CASE REPORT

A 12-year-old male child presented to our Out Patient Department on 5th June 2022 with the chief complaint of mass in the right eye for the last 8-10 days as seen in Figure 1. The pain was dull aching in nature associated with redness, watering, and foreign body sensation. There is a history of lime chemical injury. There is no history of any ocular disease or surgery. There is no history of other systemic diseases.

### Examination of the right eye

- Visual acuity: 6/18, N/6
- Pupil: Normal
- Colour vision: Normal
- Eyelid: Adhesion of the lower lid at the center.
- Eyelashes: Normal
- Palpebral aperture: Normal

Conjunctiva: Congested with the adhesion of bulbar and palpebral conjunctiva at the inferior fornix.

- Sclera: Normal
- Iris: Normal

---

\*Resident Doctor, \*\*Associate Professor, \*\*\*Senior Professor  
Department of Ophthalmology, JLN Medical College, Ajmer.

### Corresponding Author:

Dr. Raghunandan Khandelwal  
Final Year Resident  
Department of Ophthalmology, JLN Medical College, Ajmer  
Mob. 9252255211  
[dr\\_raghusms@rediffmail.com](mailto:dr_raghusms@rediffmail.com)

- Anterior chamber: Normal
- Lens: Clear

Extra ocular movements were partially restricted due to adhesion.

- Nasolacrimal duct : patent
- IOP: 14.6 mm Hg
- Fundus: WNL

#### Investigations

- Routine CBC & Blood examination: WNL
- Liver function tests: WNL
- Renal function test: WNL
- HBsAg test: negative
- HIV test: negative

Based upon the clinical and diagnostic workup, a provisional diagnosis of partial anterior symblepharon due to chemical injury was made.

#### MANAGEMENT

The patient was initially managed conservatively with antibiotic eye drops and lubricants and planned for symblepharonectomy with auto conjunctival graft

placement. Lid traction sutures were applied for adequate exposure. The conjunctival incision was made along the ends of the symblepharon and undermined from Tenon's capsule to allow the conjunctiva to retract to its normal anatomical position. The sub conjunctival fibrous tissue was excised to the maximum extent possible, and all adhesion was released. Ocular surface reconstruction was done by auto conjunctival graft to cover the bare sclera, reform the fornix, and cover the denuded palpebral conjunctiva up to the edge of the recessed symblepharon conjunctiva.

#### DISCUSSION

The intense inflammatory process due to chemical trauma will cause a progressive cicatricial process, which will limit the movement of the eyeball due to the formation of symblepharon. Treatment with symblepharonectomy alone is will result in repeated symblepharon because there is still contact between the rough surface of the eyeball and the eyelid, so to prevent it sealer is needed. Use of auto conjunctival graft to cover the bare sclera and conformer after symblepharonectomy is a cheap and simple way to form a barrier that prevents reattachment of the eyeball and eyelids<sup>5</sup>. Visual improvement was noted on postoperative day 1 and subsequent follow-up after 2 weeks with good cosmetic consideration.



Fig. 1 Preoperative



Fig. 2 Post operative

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